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hybridizing the labeled, amplified DNA products to the probe on the solid support such that the second strand hybridizes to the probe on the solid support, thereby forming prepared samples for analysis.

REMARKS

The Amendments

Claims 1 and 23 have been amended to recite expressly certain aspects of the invention which were previously implicit. Both claims now recite that a primer pair is used for amplification, not just a single primer. This unambiguously distinguishes the amplification step from mere primer extension. Both claims also now recite that the strand of the amplification product which comprises a portion complementary to the probe hybridizes to the probe. Support for these amendments is found, *inter alia*, in Figure 1. No new matter has been added to either of the claims. This amendment was not made sooner because it was required to address arguments which first appeared in the Final Office Action. In particular, at paragraph four the Office Action alleges that the choice of a primer sequence which is identical or complementary to the probe sequence is a mere substitution of equivalents. The amendments demonstrate, however, the non-equivalence of the two possible complementarities of the primer.

The Rejection of Claims 1, 5, and 7 Under 35 U.S.C. § 103(a)

Claims 1, 5, and 7 are rejected as obvious over Vary et al. Vary is cited as teaching a method of sequence-specific primer elongation in which the primer comprises:

> a 3' portion that is complementary to a region of DNA containing a polymorphic locus; and

a 5' portion containing a portion that is complementary to a probe on a solid support and not complementary to the region of DNA.

The rejection is respectfully traversed.

The Office Action states that Vary differs from the method of claim 1 only in the 5' primer sequence (page 3, lines 3-4). The Vary primer sequence is complementary to the probe on a solid support, whereas the analogous sequence in the subject claims is identical to all or part of the probe. There is, however, another important difference as well. The subject claims call for amplification using a pair of primers, but Vary teaches merely an elongation of a single primer. This difference is highly significant because it leads to a different result.

The use of a primer sequence identical to all or part of the probe sequence yields a more sensitive and reliable determination than previous methods. Such primers assure that any unlabeled primer, *i.e.*, primer which is not extended, will not compete for binding of label to the probe. According to Vary's method, however, unextended primer left in the reaction mixture competes with extended primer for binding to the probe. This interferes with quantification and analysis of the results by reducing the amount of label binding to the probe. To avoid this problem Vary's method requires a purification of unextended primer. This step is unnecessary in the claimed method.

One could not have merely changed Vary's method by substituting a primer portion which is complementary to a probe for one which is identical to the probe. Extension of a primer containing an identical sequence according to Vary's method would not yield a product which binds to the probe. Primer extension yields just one strand and that strand, because it contains a probe-identical sequence, would not bind to the probe. For binding to the probe to occur where the primer contains an identical sequence, double-stranded amplification of the primer sequence

must be performed, resulting in the synthesis of a sequence complementary to the probe. However, Vary does not teach an amplification step. Vary teaches primer elongation. Elongation alone will not produce complementary copies of a probe sequence where, as taught by Vary, the primer contains a portion identical to the probe sequence. Without a product which is complementary to all or part of the probe, the product cannot be detected. Thus, modification of the Vary method to include a primer portion identical to all or part of the probe would result in a non-functional method that produces no detectable product.

Further, claim 1 has been amended to clarify the role of certain components of an amplification protocol. The claims now recite the first and second primers of the primer pair and the first and second strands of the amplification product. Vary does not teach the use of these components because Vary does not use amplification. Vary does not teach a primer pair that includes a second primer. Neither does Vary teach the second product strand, which comprises a 5' portion complementary to all or part of the probe. The second product strand of the subject claims is produced by amplification, but it is not produced by primer extension.

Vary cannot be construed as teaching the invention of the subject claims with merely a routine substitution of "equivalent" method steps. The use of primer portions which are identical to a probe is not equivalent to the use of primer portions which are complementary because quite different results would be achieved if such primers are used in the primer elongation method of Vary. In order to achieve the same result as the method of the subject claims, the Vary method wound require multiple changes involving inventive skill. Therefore, the subject claims are not obvious over Vary, and withdrawal of this rejection is respectfully requested.

The Rejection of Claims 2-4, 6, 8, and 10-16 Under 35 U.S.C. § 103(a)

Claims 2-4, 6, 8, and 10-16[1] are rejected as obvious over Vary in view of Brown, Maniatas, and Hames. This rejection is respectfully traversed.

Claims 2-4, 6, 8, and 10-13 recite a variety of different labeling methods which are not taught by Vary. Maniatas and Hames are cited as teaching the 3' labeling of nucleotides using terminal transferase. Brown is cited as teaching the detection of a fluorescent label or radiolabel in microarrays of nucleotides hybridized to probes. Hames is cited as teaching the use of enzymatically labeled nucleotides. Claim 16 recites the use of a high density array as the solid support. Brown is cited as teaching high density arrays of oligonucleotides on a solid support.

The cited references fail to teach each element of the subject claims. Thus, even if properly combined, *arguendo*, the references would fail to make a prima facie case of obviousness. Vary is defective in not teaching either a primer region which is identical to all or part of the probe or the use of amplification driven by a pair of primers. Vary does not teach first and second primers of a pair, nor does Vary teach first and second strands of an amplification product. These aspects of the claims could not have been provided by routine choices of one of skill in the art, as discussed above.

The Office Action does not discuss several important elements of claims 10-13 and does not identify which teachings of which reference allegedly provides those elements. Claim 10 recites the determination of a ratio of different nucleotides at a polymorphic locus. Claim 11 recites the determination of the same ratio with simultaneous determination of the different nucleotides. Claim 12 recites the use of a sample comprising DNA from two or more

[1] No explanation is provided in this section of the Office Action for the rejection of claims 14 and 15, which are the subject of a separate rejection. Therefore, this rejection is presumed to refer only to claims 2-4, 6, 8, 10-13, and 16.

individuals. And claim 13 recites the amplification from two or more polymorphic loci simultaneously. It is unclear whether these elements are to be found in Vary, Brown, Maniatas, Hames, or other references not cited. If these elements are not taught by any cited reference then the rejection of these claims must fail.

Because the combination of all four cited references does not teach or suggest important elements of the subject claims, the withdrawal of this rejection is respectfully requested.

The Rejection of Claim 9 Under 35 U.S.C. § 103(a)

Claim 9 is rejected as obvious over Vary in view of Okayama. Claim 9 recites the use of two primer pairs in the amplification step so that the presence or absence of two different nucleotides at the same polymorphic locus can be detected at distinct locations on a solid support. Vary does not teach the use of any pairs of primers. Okayama teaches the use of two primer pairs to determine a nucleotide at a polymorphic locus. The rejection is respectfully traversed.

Again, the cited references even if combinable, *arguendo*, do not teach or suggest the method of the subject claim because several elements of the claim are not taught by either reference. Neither Vary nor Okayama teach a primer comprising a region that is identical to all or part of a probe on a solid support. Vary does not teach an amplification step. Okayama teaches amplification using two pairs of primers. However, Okayama teaches detection of the results by agarose gel electrophoresis with ethidium bromide staining. While Vary does teach the use of a tagged primer, with binding of the tag to a probe on a solid support, Vary does not teach a tag with appropriate complementarity to achieve the benefit of the method of the subject claims, as discussed above. Thus, Okayama does not teach, either alone or in combination with

Vary, the amplification step of claim 9, wherein a primer comprising a region identical to all or part of a probe on a solid support is used to produce a product that is complementary to all or part of the probe. The withdrawal of this rejection is therefore requested.

The Rejection of Claims 14 and 15 Under 35 U.S.C. § 103(a)

Claims 14 and 15 are rejected as obvious over Vary in view of Lockhart. Claims 14 and 15 recite the use of beads or a microtiter dish as the solid support in the method of claim 1. Vary fails according to the Office Action to teach the use of beads or a microtiter dish as the solid support. Lockhart is cited as teaching the use of beads and a microtiter dish as a solid support for oligonucleotide probes. The rejection is respectfully traversed.

As before, the subject claims are not rendered obvious by the alleged combination of Vary and Lockhart because the combination even if proper fails to teach several elements of the claims. Vary is defective in not teaching either the primer region which is identical to all or part of the probe or the use of amplification driven by a pair of primers. Vary also does not teach the first and second primers of the pair, nor does Vary teach the first and second strands of the amplification product. Lockhart's teaching of solid support materials does not remedy these defects. Withdrawal of this rejection is therefore requested.

The Rejection of Claims 23, 27, and 29 Under 35 U.S.C. § 103(a)

Claims 23, 27, and 29 are rejected as obvious over Vary et al. The subject claims recite a method to prepare samples for analysis. The method comprises the steps of amplifying, labeling, and hybridizing of claim 1. As discussed above, Vary did not render claim 1 obvious because Vary did not teach the step of amplifying, including the primers and products involved. Since

claim 23 employs the same step of amplifying as claim 1, it follows that Vary cannot render claim 23 obvious for the same reasons it could not render claim 1 obvious. In addition Vary does not teach hybridization of an amplified product to a probe on a solid support. Vary does not teach or suggest several elements of the subject claims, including a step of amplifying, a pair of primers, a first primer with a region identical to all or part of the probe, and a second product strand with a region complementary to all or part of the probe. The elements not taught by Vary are not mere equivalents that would have been routinely substituted into the simple primer extension reaction of Vary.

For the reasons discussed above, this rejection should be withdrawn.

The Rejection of Claims 24-26, 28 and 30 Under 35 U.S.C. § 103(a)

Claims 24-26, 28, and 30 are rejected as obvious over Vary in view of Brown, Maniatas, and Hames. The subject claims recite various labeling techniques not taught by Vary, but allegedly found in the teachings of Brown, Maniatas, or Hames. The rejection is respectfully traversed.

This rejection parallels the rejection of claims 2-4, 6, and 8 over the same combination of references, and it fails for the same reasons, as discussed above. Briefly, Vary does not teach or suggest several elements of the subject claims, including a step of amplifying, a pair of primers, a first primer with a region identical to all or part of the probe, and a second product strand with a region complementary to all or part of the probe. It would not have been obvious to substitute the elements taught by Vary into the simple primer extension reaction of Vary. As discussed above, such a simple substitution would not have resulted in a functional method. Since the deficiencies of Vary are not remedied by either Brown, Maniatas, Hames, or any combination

thereof, the subject claims are not obvious over the cited combination of references and this rejection should be withdrawn.

The Rejection of Claims 31-35 Under 35 U.S.C. § 103(a)

Claims 31-35 are rejected as obvious over Vary in view of Okayama. The rejection is respectfully traversed.

Claims 31 and 32 recite the use of two primer pairs in the amplification step so that the presence or absence of two different nucleotides at the same polymorphic locus can be detected at distinct locations on a solid support. Vary does not teach the use of any pairs of primers or an amplification step. Okayama is cited as teaching the use of two primer pairs in an allele-specific amplification method to determine a nucleotide at a polymorphic locus. The alleged combination of Vary and Okayama does not teach or suggest several elements of claims 31 and 32. Neither Vary nor Okayama teach a primer comprising a region that is identical to all or part of a probe on a solid support. Therefore, neither Vary, Okayama, nor their combination teaches the amplification step of claim 31 or 32, wherein a primer comprising a region identical to all or part of a probe on a solid support is used to produce a product that is complementary to all or part of the probe.

Claim 33 recites the determination of a ratio of nucleotides at two or more polymorphic loci simultaneously using the method of claim 32. At page 108, Okayama teaches the simultaneous determination of two alleles (Z and M) at a single polymorphic locus. However, Okayama does not teach or suggest the simultaneous determination of alleles at more than one locus. Therefore, the combination of Vary and Okayama fails to suggest a further element in

claim 33, which is the use of two or more primer pairs to simultaneously amplify two or more different polymorphic loci.

Claim 34 recites the method of claim 23 wherein the sample comprises DNA from two or more individuals. Okayama teaches comparison of the results obtained from parallel analysis of different individuals (page 109). However, Okayama does not teach the analysis of a single combined sample derived from two or more individuals. Since these two approaches produce different types of results and are not at all equivalent, it is apparent that another element of claim 34 is missing in the combined teachings of Vary and Okayama.

Claim 35 recites the method of claim 23 wherein two or more distinct polymorphic loci are amplified in a single reaction mixture. Okayama teaches the simultaneous determination of two alleles (Z and M) at a single polymorphic locus and also teaches comparison of parallel tests of different individuals. However, Okayama does not teach simultaneous amplification at two or more polymorphic loci. Figure 2 at page 109 of Okayama provides information on different loci, but amplification and analysis are performed at only one locus at a time. Thus, Okayama fails to suggest another element of claim 35.

From the above discussion, it is clear that neither Vary, Okayama, nor their combination teaches or suggests all the elements of any of the subject claims. Therefore, withdrawal of the rejection is requested.

The Rejection of Claims 36-38 Under 35 U.S.C. § 103(a)

Claims 36-38 are rejected as obvious over Vary in view of Lockhart. The subject claims recite the use of beads, a microtiter dish, or a high density array as the solid support for the probe. Vary does not teach these solid supports, which are allegedly provided by Lockhart. The rejection is traversed.

The subject claims are not rendered obvious by the alleged combination of Vary and Lockhart because the combination fails to teach several elements of the claims. Vary is defective in not teaching either the primer region which is identical to all or part of the probe or the use of amplification driven by a pair of primers. Vary also does not teach the first and second primers of the pair, nor does Vary teach the first and second strands of the amplification product. Lockhart's teaching of solid support materials does not remedy these defects. Therefore, this rejection should be withdrawn.

Allowance of all pending claims is respectfully requested.

Respectfully submitted,

Date: December 1, 2000

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